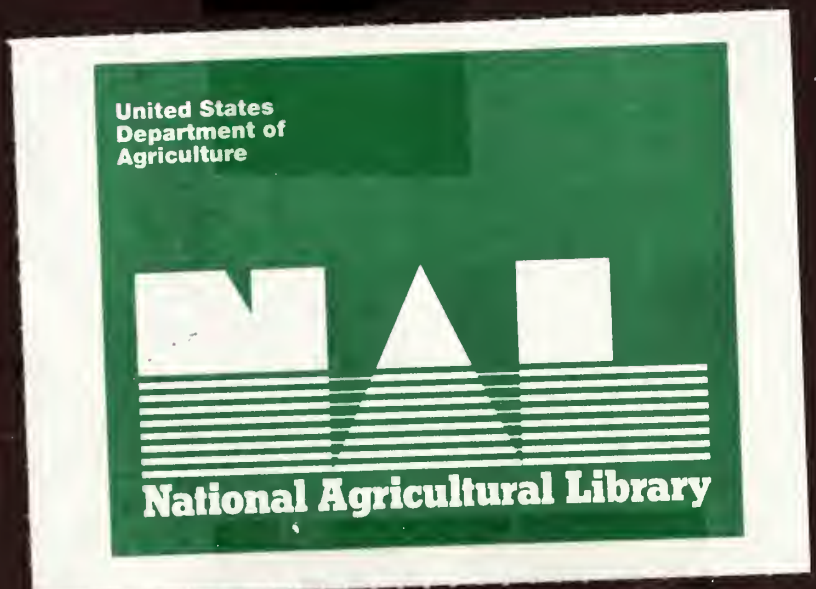
REPORT

OF THE

SALMONELLA ENTERITIDIS

TASK FORCE FOR RESEARCH

SEPTEMBER 1988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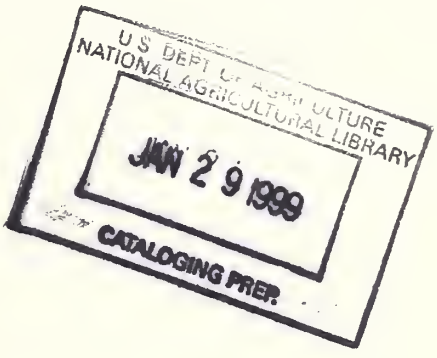

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SALMONELLA ENTERITIDIS

EXECUTIVE SUMMARY

Salmonellosis due to Salmonella enteritidis has increased steadily beginning in the late 1970's in the New England and Mid Atlantic States as a cause of gastroenteritis in humans. The Centers for Disease Control at Atlanta, Georgia, have found that 77 percent of outbreaks of Salmonella enteritidis with identified food vehicle were associated with eggs. Egg-containing foods have not been a problem as the cause of salmonellosis since the institution of the Egg Products Inspection Act of 1970 and other changes in the egg industry have led to the near disappearance of egg-associated salmonellosis in this country. Recent data indicates that the Salmonella enteritidis organism can be egg associated and may not be controlled by current egg shell sanitizing procedures. Salmonellae can contaminate the interior of the egg by penetrating the shell or may contaminate the egg contents inside the hen before or during shell formation. The latter, which includes transovarian transmission is suggested strongly by several epidemiologic observations, although the shedding rate from infected hen to the egg is apparently very low. Inferences are that the organism is shed in a very low concentration and probably requires amplification through abuse of proper holding temperature during storage, transportation or preparation at some point before consumption.

This emerging public health issue has not been confined to the United States. There has been a dramatic increase of Salmonella enteritidis in Canada and an even greater increase (five fold) in cases in Europe. There are indications that the change could be due, at least in part, to a change in virulence of the organism. Yet the infection in adult birds does not frequently cause a severe clinical infection. There is information in Europe that the disease in younger birds, broiler age, may be much more severe, especially if due to infection by a specific phage type of Salmonella enteritidis, phage type 4. We do not know the potential impact of Salmonella enteritidis to the broiler and the turkey and other sectors of the poultry and livestock industry.

Because of the human deaths and the public attention to the problem, there is potential for litigation or liability losses in addition to severe adverse market effects to the layer industry.

Control strategies must be put in place before much is known or fully understood about Salmonella enteritidis. Research must catch up to the needs of the producers and regulatory and state health officials and others who are making decisions regarding control and prevention strategies.

SALMONELLA ENTERITIDIS

Define the Situation:

Salmonellosis is the most commonly reported bacterial cause of gastroenteritis in the country, causing an estimated 4 million infections per year and over 1,000 deaths. Most infections are gastroenteritis; however, the immunocompromised, the elderly, and those exposed to very large doses of the bacteria are at risk for bacteremia, deep tissue infection, and death.

Salmonellae are widely distributed throughout the animal kingdom and can infect humans through a wide variety of different vehicles, most often foods of animal origin.

The total human cases of Salmonella enteritidis have been steadily increasing (Table A). There has been a steady increase of Salmonella enteritidis in the New England States beginning in the late 1970's. In 1982, the isolation rate of Salmonella enteritidis began to increase in the Mid Atlantic Region and jumped more dramatically in 1984. Now, more recently, the South Atlantic Region is beginning to show an upward drift. The Centers for Disease Control, (CDC) found that 43 percent of the Salmonella enteritidis outbreaks were associated with an egg vehicle in a retrospective review of outbreaks reported from 1973 to 1984. In more intensively investigated outbreaks that occured in 1985 to 1987 in the Northeastern United States, 77 percent with identified food vehicles were associated with eggs or egg containing dishes.

Egg-containing foods were once a major source of salmonellosis. However, the institution of the Egg Products Inspection Act of 1970 and other changes in the egg industry were attended by the near disappearance of egg-associated salmonellosis in this country.

Recent data suggest that a serotype of Salmonella with a rapidly increasing incidence, Salmonella enteritidis, can be egg-associated and may not be controlled by current procedures that focus on sanitizing egg shells to prevent penetration from the environment through the shell.

Some traditional methods of consuming eggs or the use of egg products are not sufficient to kill Salmonella enteritidis. These include eating "sunny side-up," fried eggs, soft-cooked scrambled eggs and custards, and the use of raw eggs in salad dressings, sauces, and other foods or drinks **(116)**.

How would the content of the Grade A shell eggs become contaminated with Salmonella? Potential routes include local soilage of the outside of the eggs with passage of Salmonella enteritidis through the shell and transovarian contamination of the egg contents inside the chicken before or while the shell is formed. Several epidemiologic observations suggest

that transovarian transmission of Salmonella enteritidis may be an important factor in our current problem. However, field data and results from experimental infections of hens with Salmonella enteritidis indicate a very low rate of infected eggs resulting from infected hens. When examining all eggs from known infected flocks, only 0.4 percent of the eggs have been found to contain Salmonella enteritidis. It has been estimated that the risk to humans posed by an infected flock is very low. Therefore, there is strong evidence for amplification of the problem due to unsafe storing, handling, and cooking of eggs and egg products.

There is evidence of differences among the Salmonella enteritidis strains, whereby some may be more invasive and more virulent than others **(114)**. Genetic fingerprinting identifies at least two different patterns among chicken isolates, but these differences have not been established among isolates collected over a period of time. Studies conducted on phage-typing show no differences when comparing isolates from the last 10 years. However, European workers have reported a dramatic increase in certain phage types. Phage-type 4 is causing high mortality in birds in Europe (CDC Report) and accounts for 50 percent of 16,000 human cases in Italy. This phage type has not been identified in the United States. As we see Salmonella enteritidis in poultry in the United States, it is not a severe clinical disease on a flock basis. Although it can cause declines in production and lesions resulting from systemic infections, this is different from the situation observed in Europe with phage-type 4, which has been reported to kill young broiler-age birds. We have not had the opportunity to observe this disease in flocks of young birds.

This emerging public health issue is not confined to Salmonella enteritidis from egg sources. There has been a steady increase of salmonellosis in the United States due to sources other than eggs, as well as a steady increase of Salmonella enteritidis in relation to other serotypes of Salmonella. There has also been a dramatic increase of Salmonella enteritidis in Canada and an even greater increase (five fold) of Salmonella enteritidis cases in Europe. The increases in Salmonella enteritidis cases from Canada indicate that no vehicle association was established.

CDC does not see a change in virulence or vehicle in the last decade. The egg association to Salmonella enteritidis has been made only recently, but as epidemiologic tracebacks are being conducted on old cases, egg-related human cases can be identified back to 1973.

Human cases are more prevalent during the summer time. This seasonal relationship has been observed in the United States and in Europe. These seasonal increases may be used circumstantially to implicate abuses in food storage and handling. There is a recognized lack of knowledge and training in many institutional food handlers.

Illinois has instituted a state-wide regulation for storage of eggs at 45 degrees F; this may eventually become a nationwide practice as more fast-food chains adopt it. According to the FDA Unicode, if eggs are handled like pasteurized milk and raw meat, the storage temperature could become 40 degrees F. Egg houses currently store the eggs at 60 degrees F.

The sanitizing of eggs must be enforced. Preliminary experimental data indicated that some virulent strains of Salmonella enteritidis can penetrate through the unbroken shell within 24 hours.

We do not know the importance of the transovarian mechanism of egg contamination to human health. However, in attempting to find a solution to the Salmonella enteritidis problem, we must start by controlling vertical transmission from parent to progeny, while evaluating the role of horizontal spread.

Assuming that vertical transmission is the means of spreading Salmonella enteritidis with the greatest public health significance, attempts must be made to start surveying the primary flocks of egg-type breeders, continuing with certification of the grandparent flocks to the multipliers, and finally the commercial layers. These approaches are recommended based on prior experiences with the eradication of a related serogroup of Salmonella, Salmonella pullorum.

In addition to surveying breeding flocks, commercial laying flocks also must be properly examinied using culture isolation and serology when they are implicated by public health tracebacks from outbreaks in consumers.

Based on Salmonella pullorum experience, we must also consider management practices to prevent horizontal transmission. This is a long-term problem with no quick-fix solutions. Among the management practices, we must assure a safe feed source with Salmonella-negative finish feeds.

Introduction or reintroduction of Salmonella enteritidis by rodents and wild birds or even by workers on poultry farms must be considered possible.

Because of the human deaths and the public attention to the problem, there is potential for litigation or liability losses in addition to severe adverse market effects to the layer industry.

We do not know the potential impact of current United States or foreign serotypes of Salmonella enteritidis on the broilers and turkeys and other sectors of the poultry and livestock industry.

Because this is a complex situation, where the egg is not the only vehicle of human infections and where the lack of severe clinical disease in layers complicates the rapid diagnosis, the solution to this problem must be addressed on a step-wise basis.

WHAT HAPPENED, WHAT CHANGED

We do not have a proven scientific explanation for the worldwide increase of Salmonella enteritidis cases. The following is a list of potential factors that may have played a role:

-- Although egg-associated Salmonella enteritidis cases have increased in the United States, there also has been an increase in all Salmonella enteritidis cases and in total salmonellosis cases as well. The accurate number of egg-associated cases is difficult to determine since the public awareness of this problem has created a bias to implicate eggs or egg products in recent Salmonella enteritidis cases.

-- There could have been a tie-in between the appearance of Salmonella enteritidis in egg-laying flocks in the United States and the increased importation of European birds.

-- There could have been an increase in susceptibility to Salmonella enteritidis in layers after they started being raised in cages (1975), thereby preventing early colonization of their guts with other competing organisms.

-- There have been changes in the conveying equipment used for the handling of eggs and the disinfection of this equipment.

-- Because of perceived occupational health risks, the use of formaldehyde for sanitizing eggs has been reduced in multiplier flocks and often replaced by chlorination. Although these changes are not across the entire industry, there have been significant changes.

-- It is possible that Salmonella enteritidis gained access to the multiplier flocks and was then spread rapidly through the laying industry. Over time, the organism may have become better adapted to the host.

-- A shift in incidence of Salmonella enteritidis cases has been observed from the New England States to the Mid Atlantic and, more recently, to the South Atlantic. This could be related to changes in bird population replacements after the avian influenza outbreak.

There is evidence of worldwide changes in the manifestations of Salmonella enteritidis infections that could be due to changes in the genetic makeup. A new phage-type (type 4) in Europe accounts for 50 percent of the cases and causes severe disease and mortality in broilers. This phage-type has not been isolated in the United States.

RESEARCH NEEDS

The following is a list of issues where research is needed. These are not in order of priority.

Diagnostic Techniques and Sampling Methods: These include both sensitive and practical serological techniques and culture isolation procedures.

We do not know how well Salmonella enteritidis competes with other Salmonellae and other organisms in media and/or tissues. This information is essential for the planning of isolation studies.

Pathogenesis: We need to understand the biology of Salmonella enteritidis in the bird, persistence and rate of shedding, vertical vs. horizontal transmission, the effects of age and breed of birds, the differences among Salmonella enteritidis strains, and the correlation of serology with various stages of infection.

How important is transovarian transmission as a means of contaminating table eggs and systemically infecting replacement chicks?

Epidemiological studies are needed to determine the role of wildlife and rodent reservoirs in infected flocks.

We need to know the effects of highly virulent strains in young chickens (<6 weeks). The general pathogenicty of Salmonella enteritidis strains for young broilers and/or adults is not known.

The significance of Salmonella enteritidis infections detected in a laying flock by routine survey is unknown. Studies are needed to determine means to identify systemically infected birds from fecal carriers. Flocks identified as positive as a result of a public health traceback may need to be handled differently from flocks found to be positive from routine surveys. Decisions on flock disposition made in the basis of serological titers and/or on the basis of fecal cultures alone could result in overkill. A total of 500,000 layers have been destroyed as a result of a public health implication. This was done without indemnification to the owners.

Information is needed on the number of infected eggs produced by Salmonella enteritidis-infected birds and/or flocks.

Research is needed on preferred procedures for sanitizing eggs and egg-handling equipment.

Work is needed for determining proper recommendations for decontamination and clean-up of premises. What is the survival time for Salmonella enteritidis in the environment?

Food Science: Information is needed on time/temperature requirements of storage, handling, and shipping of eggs. We also need information on cooking time and temperature and critical control points to assure killing of Salmonella enteritidis in eggs and other foods.

We need to increase training and develop new methodologies for training kitchen help and institutional food handlers.

Research is needed on practical ways of identifying source and laying date for eggs.

Control, Prevention, and Treatment: Research is needed to determine the efficacy of different types of vaccines (polyvalent, monovalent, live attenuated, killed), adjuvants, and delivery systems for protection against Salmonella enteritidis infection.

Means for prevention: Medications, competitive exclusion, use of anti-Salmonella feed additives, and pelleting of feed must be investigated.

In order to expedite a control program, we need to improve methods for traceback and develop alternatives to slaughter. Protocols for recertification of flocks as negative and safe for egg production must be developed.

There is a need for improved informational exchange between the USDA, State Departments of agriculture, Industry, Universities, and Public Health Agencies. Improved quality and timeliness of distribution of human outbreak data to the industry and research community are needed.

Establishment of Strain-Typing Centers: It is essential for a rapid assessment of virulence and for effective epidemiological studies to establish proper strain-typing laboratories. Pathogenic Salmonella enteritidis needs to be characterized using various parameters such as phage-typing, plasmid analysis, and genomic fingerprinting. Information gathered from these studies should help identify the necessary markers for virulence.

Current research activities are noted in Table B.

SUMMARY OF RESEARCH NEEDS OF HIGHEST PRIORITY

Short Term

- Develop rapid, sensitive, and practical serologic and microbiologic techniques to identify infected flocks and to differentiate systemically infected hens from intestinal carriers.

- Determine the relative public health significance of vertical versus horizontal spread in flocks.

- Develop organizational structures that will insure the coordination and exchange of information.

- Establish appropriate time/temperature guidelines for handling, shipping, storing and cooking eggs.

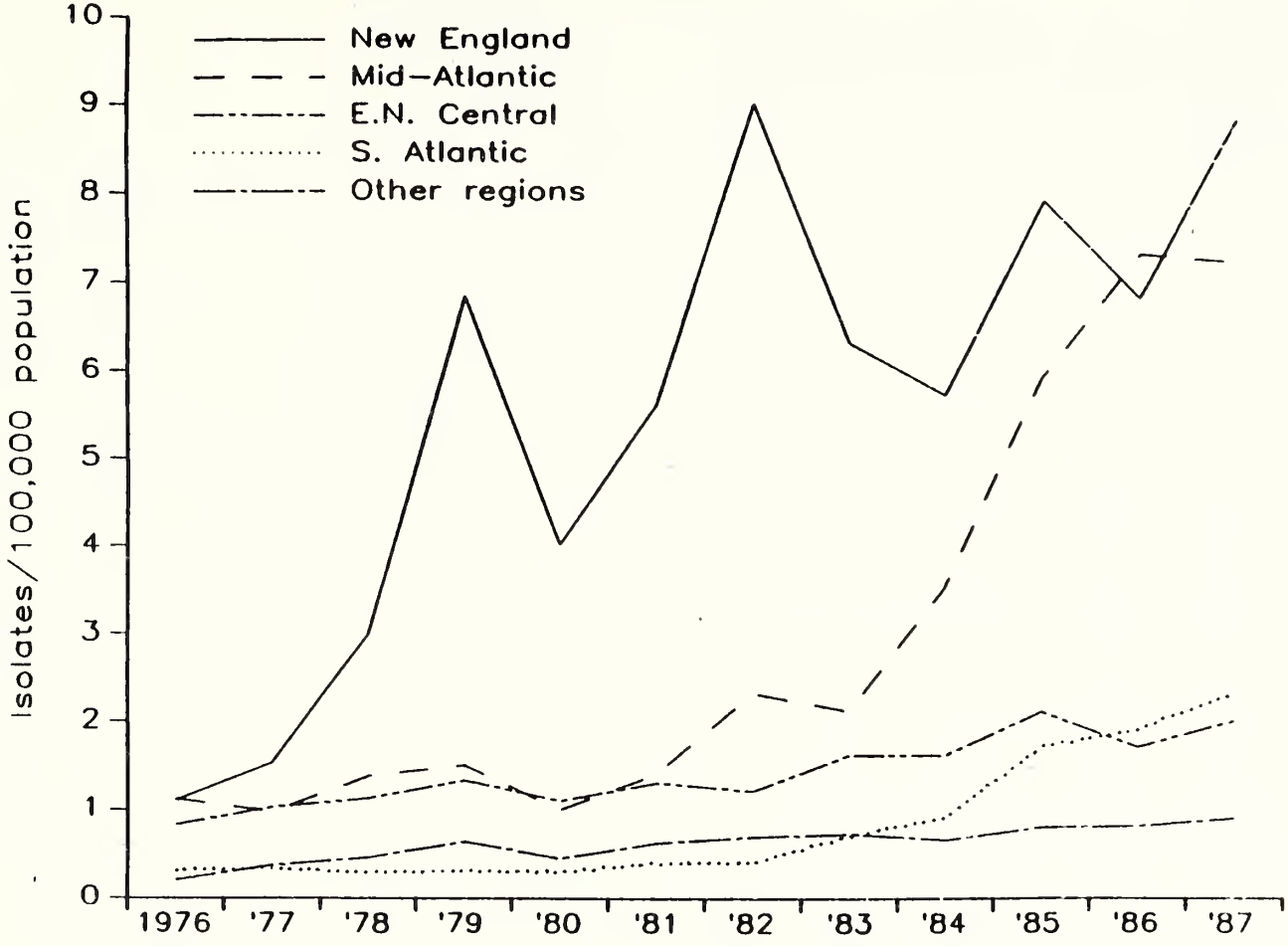
- Develop training videos for food handlers, etc., for use by Extension Service, Institute of Food Technology and Home Economists.

- Determine the proper decontamination and clean-up procedures for premises, and Investigate the survival time for Salmonella enteritidis in the environment.

Long Term

- Develop more practical sampling and sensitive diagnostic techniques.
- Continue to type strains and correlate strains to virulence in humans.
- Further define pathogenesis, biology of Salmonella enteritidis in poultry.
- Continue exploration of vaccine use.
- Study and evaluate the potential of competitive exclusion in control measures.
- Continue to standardize the following:
    - o Diagnostic tests and reagents.
    - o Methods to determine virulence of the organism.
    - o Methods to evaluate efficacy of vaccine and other intervention strategies.

Salmonella enteritidis isolation rate, by region, United States, 1976–1987



Source: CDC

SALMONELLA ENTERITIDIS - CURRENT RESEARCH EFFORT

Problems	Institution or Organization														
	UN of PA	PA ST UN	PA Dept Agr.	Cornell	UN of MD	UN of ME	WA UN	UN of MN	MD Dept Agr.	UN of MO	UN of ID	ARS	CDC	FDA	NVSL
I Transmission of Salmonella enteritidis into Eggs and Bird to Bird Within Flocks	X	X		X	X	X	X					X			
II Persistence of Salmonella enteritidis in Eggs from Infected Flocks	X	X		X								X			
III More Rapid and Sensitive Diagnostic Tests for Detection of Infected Flocks	X	X	X		X			X	X	X		X			
IV Need for Vaccine and Diagnostic Antigens							X	X			X				
V Need to Define Pathogenesis of Salmonella enteritidis in the Avian Host	X	X				X	X					X			
VI Thermal Death Time				X										X	
VII Genomic Fingerprinting, Phage Typing, Plasmid Analysis						X		X					X		
VIII Research Support, Reagents, Antigens, Reference Materials															X
Full Time Equivalents	4.5	3.0	0.2	2.0	0.3	2.2	0.7	2.5	0.5	0.5	0.5	2.0	0.3	0.4	0.2

LITERATURE REVIEW

INCIDENCE INTERNATIONALLY

From 1976 to 1986, reported Salmonella enteritidis infections (53) increased more than sixfold in the Northeastern United States. From January 1985 to May 1987, sixty-five foodborne outbreaks of Salmonella enteritidis were reported in the Northeast that were associated with 2119 cases and 11 deaths. Twenty-seven (77%) of the 35 outbreaks with identified food vehicles were caused by Grade A shell eggs or foods that contained such eggs. National data from 1973 to 1984 showed that Salmonella enteritidis outbreaks (44%) were more commonly associated with egg-containing foods than were outbreaks of other Salmonella serotypes (18%). Reflecting the geographic distribution of human illness, cultures of bulk raw eggs from pasteurization plants in the Northeast more frequently yielded Salmonella enteritidis (10%) than did eggs from other regions of the United States (0%). The epidemic rise in Salmonella enteritidis infections due to Grade A shell eggs is unlike past problems of salmonellosis associated with cracked or soiled eggs and raises the possibility of trans-ovarian contamination of eggs with Salmonella enteritidis. New techniques may therefore be needed to control resurgent egg-associated salmonellosis in the United States. There were increasing numbers of isolations from animals and human cases in many parts of the world.

There were increasing numbers of isolates of salmonella domestic animals (54) from 1976 to 1980 over that observed 1970-75. Salmonella enteritidis was among the most common serotypes noted in Bulgaria. During the same period Salmonella isolates from mixed feed decreased 8.1% to 2.5%. There was a notable increase (20) of Salmonella enteritidis strains in Spain (27% in 1980 to 66% in 1985). Workers using 6 bacteriophages typed 1500 strains, and there were only 1.4% non-typable. In a Russian study (38) of 1806 flocks, 1.7% of chickens yielded Salmonella. In 1978, there were in Poland 4 large epidemics (44) of Salmonella food poisoning: 822 cases in Wroclaw province of Salmonella enteritidis infection from cream and milk, 701 cases in Suwalki province, 501 such cases, in Katowice province, and 492 cases in Legnica province of Salmonella enteritidis infection also from ice cream.

Studies (48) documented relatively low rate of isolation of Salmonella enteritidis (2%) from poultry in Czechoslovakia. However (60), in a ten year study (1971-1981) Salmonella enteritidis in turkeys was reported to be the 3rd most common isolate in Czechoslovakia. Salmonella enteritidis (49), was noted as among the common Salmonellae (16%) from animals in Rumania in 1971-1980. Salmonella enteritidis (51) was documented as among the more common Salmonella isolated (13.4%) from poultry in Croatia in 1983.

Another study (56), documented Salmonella enteritidis as an infrequent isolate from a variety of animals cultured in Greece (5 of 75 isolates) in 1983. In England (42) in a survey done retrospectively for the period 1968-74 of 1744 isolates from poultry only 150 were Salmonella enteritidis.

Salmonella enteritidis infection (50) was found to be frequent in hedgehogs in Switzerland in 1983.

In Brazil in 1976, Salmonellae (43) were recovered from 60 of 300 poultry carcasses, namely 23% of 150 carcasses from one farm, and 17% of 150 carcasses from another farm. Serotypes comprised anatum, typhimurium and enteritidis on the first farm and oranienburg, haardt, typhimurium and anatum on the second. Much the same serotypes were also recovered from feed and poultry house litter.

All of the Salmonella enteritidis strains (58) isolated from diseased animals (61 strains) and from beef (2 strains) in Japan and in West Germany (1 strain), except for 2 strains isolated from ducks, harbored either a 36-megadalton (Md) plasmid alone or in combination with several other plasmids of different sizes. It is likely that these 36-Md plasmids from various Salmonella enteritidis strains were derived from the same origin. The authors also suggested that this plasmid is native to Salmonella enteritidis. The strains without the 36-Md plasmid showed less virulence compared to a wild-type strain harboring the 36-Md plasmid, suggesting that this 36-Md plasmid might be associated with virulence for mice.

Data on incidence of Salmonella (55) in livestock and poultry in Japan indicated that Salmonella entertidis was more frequently recovered from cattle than chickens. In another study in Japan (21), Salmonella typhimurium and Salmonella enteritidis were isolated from cattle frequently. More than 70% of those isolated were resistant to certain antibiotics. In 1982, isolation of Salmonella enteritidis (63) from eggs was reported from Pakistan. Salmonella enteritidis accounted for 2.7% of the total isolates.

In 1984, eleven of 60 chickens flocks (33) in Canada contained Salmonella enteritidis in their colons upon slaughter. Workers at Cornell University found duck eggs (17) to be a source of Salmonella enteritidis in 1985.

TRANSMISSIBILITY, SPREAD AND RESPONSE IN POULTRY

Salmonella enteritidis (2) was demonstrated by workers at the University of Massachusetts in 1974 to translocate from intestine to internal organs of chicks. This supports horizontal transmission of Salmonella enteritidis. In England, in 1978, (3) Salmonella enteritidis

was demonstrated by electron microscopy to be able to enter a chick's body following ingestion; again supporting horizontal transmission. Workers in England (6) suggested that the passage of Salmonella enteritidis and Salmonella thompson from the gut of chicks to the lamina propria is primarily the result of capture and transport by host macrophages. English workers (7) also found that Salmonella enteritidis can penetrate the epithelium of the gut of one day old chicks as demonstrated by light and electron microscopy. Findings in a study in 1983 (10) strongly support the hypothesis that when bacteria contain polysaccharide it activates complement efficiently, the bacteria will be phagocytosed, whereas if the polysaccharide activates complement poorly, the bacteria escape ingestion and may cause disease.

University of Massachusetts workers (18) found that chicks infected at one day old yielded the highest level of positive cloacal swab cultures, whereas exposure of older birds have maximal serologic titers.

In the United Kingdom (40) in an outbreak of turkey-borne food poisoning between 1975 and 1976, there were 185 human cases. All episodes were associated with turkeys or turkey poults from one breeding and rearing establishment. Salmonella enteritidis was involved in the first outbreaks, and Salmonella hadar in later cases. These studies showed (a) that neither Salmonella enteritidis nor Salmonella hadar were causing significant losses in turkeys, (b) that although many birds may be infected without clinical disease, they may become excreters, and (c) that although both Salmonellae were known to be present on the farm, cloacal or rectal swabs and litter samples were largely unreliable for their detection because excretion was intermittent. In 1979, workers at the University of Massachusetts (45) found that chicks infected when one day old have the highest level of positive cloacal swab cultures, whereas exposure of older birds gave maximal serological titres. Recovery of Salmonella from environmental samples is dependent on a number of factors, including excretion rate by the population and survival rate of Salmonellae in the environment. Serological titres persisted after Salmonellae could no longer be isolated from cloacal swabs or environmental samples.

Outbreaks of Salmonella enteritidis infection in broiler chicks (52) first began in the UK in 1987. In an affected flock in East Anglia up to 5% of chicks failed to grow and were culled. Post-mortem examination of affected 2-week-old chicks showed a toxic indurated yolk sac remnant, pericarditis and occasional necrotic foci and petechiae in the liver. Salmonella enteritidis phage type 4 was isolated from the heart. The infection appeared to have been transmitted vertically, probably as a result of transovarian infection. Pericarditis was subsequently seen in a small number of broilers at the time of slaughter. The isolation (62) of Salmonella enteritidis from internal organs from broilers was done by Polish workers in 1986. They also document the

isolation of this Salmonella serotype from internal organs of geese. In a study (39) in Bangladesh in 1976, Salmonella enteritidis was isolated in 4 of 50 dead embryos, typhimurium in 3, pullorum in 2. No isolations were made from fresh eggs. The flock of origin had low hatchability problems.

In 1977 (23) work done in Israel found that eight of 15 species of wild Israel birds were carriers of Salmonella including Salmonella enteritidis. These birds may be a source of infection for poultry. Salmonella enteritidis (46) has been recovered from wild Sandhill cranes in 1977 by workers at the University of Wisconsin.

ENTEROTOXINS, CYTOTOXINS

In India (4) workers found that Salmonella enteritidis has the capability to produce hazardous levels of enterotoxin under appropriate conditions. In the Netherlands (1) workers found in 1982 that the high molecular weight enterotoxin protein and the invasive properties of Salmonellae (including Salmonella enteritidis) appear to play an important role in pathogenesis. At the University of Wisconsin (5) it was determined that an enterotoxin complexed with the endotoxin of Salmonella enteritidis may be involved in the pathogenesis. The character of the enterotoxin has not been determined.

In 1984 (11) a study determined that Salmonella cytotoxin present in cell lysates inhibited protein synthesis in both Vero cells and isolated rabbit intestinal epithelial cells. A similar pattern of protein synthesis inhibition was observed when isolated epithelial cells from normal rabbit intestine were exposed to the Salmonella cell lysate. The inhibited protein synthesis in the intestinal cells provides a molecular basis for the cellular damage caused by Salmonella cytotoxin during experimental salmonellosis. In 1983 (12) workers isolated Salmonella enteritidis and Salmonella Thompson from humans which produced a toxin unrelated to cholerae toxin.

DIAGNOSTICS, INCLUDING VIRULENCE MARKERS

For many years phage typing has proved invaluable (26) in epidemiological studies on Salmonella typhi, Salmonella pratyphi a and b, Salmonella typhimurium and a few other serotypes. Workers in England (24) reported on a phage typing system to differentiate 27 types of Salmonella enteritidis using 10 typing phages.

A study in France and Spain (25) described efforts to establish phage-typing of Salmonella enteritidis as an epidemiological tool to deal with great increase in Salmonella enteritidis seen in Spain from 1980-85.

A study coordinated by CDC in 1987 (120) compared Salmonella enteritidis phage types using the phage typing system at the University of Maine and the system of typing by the Reference Laboratory in England. Most strains tested fell into phage type A in this Maine system, with fewer types B, E, G, and Q. Predominant phage types with the English system were 13a, 14 and 8; the two systems did not correlate perfectly with each other or with CDC's plasmid profile patterns.

Using a different system in Hungary in a human study (27) human strains were typable in 99.4% and they belonged into 21 phage types. Five phage types (1, 4, 7, 16 and 17) were more frequent than 1%. Phage type 7 predominated among the strains isolated between 1976 and 1980.

Workers in Bulgaria (34) found that susceptibility to bacteriophage O-1 was useful as an adjunct supplemental method in identifying Salmonella serotypes.

In Hungary (13) there was a change in the prevalence of phage types from 1980-1981, as phage type 7 was replaced by phage type 1. The date of the change in the predominance of phage types coincided with the considerable increase of Salmonella enteritidis isolates; the number of isolates was nearly fivefold in 1980 compared to 1976. Phage type 7 frequency in the first period proved to be homogeneous. It was obvious in the case of county Tolna that the source of infection was contaminated egg and baby chicken (phage type 1).

Phage-type 4 Salmonella enteritidis in British broilers again using the English system (8) was implicated as the pathogen in two-week-old chicks with lesions possibly at the time of slaughter. Transovarian transmission was suspected.

Workers at the Department of Medicine (16), University of California, San Diego, found various (5) Endonuclease digestion patterns for Salmonella enteritidis as possible markers for virulence.

French workers (28) found that plasmid groups may contribute to the pathogenic potential of Salmonella serotypes that are known to be host adapted or potentially so.

Workers in Japan (29) theorized that a 36-MD plasmid, apparently native to Salmonella enteritidis and derived from the same origin, might be associated with the virulence of certain strains of Salmonella enteritidis for mice.

In France it was reported that a group of plasmids (30) has been identified that may be responsible for the pathogenic potential of Salmonella enteritidis.

In a study in 1985 it was determined (36) that the influence of species specific plasmids on virulence was documented for Salmonella typhimurium, Salmonella enteritidis, Salmonella dublin and Salmonella choleraesuis in mice. Plasmids of distinctive molecular weights appear to influence the number of organisms required to establish an oral LD50. Plasmid carrying strains invade the liver and are resistant to host defenses.

In a study in Canada (57) in 1987, a collection of 185 isolates of 34 serovars of Salmonella from avian sources was examined for plasmids, drug resistance, biochemical properties, serum resistance, and virulence. No serovars other than Salmonella enteritidis, Salmonella typhimurium, and Salmonella heidelberg showed evidence of serovar-associated plasmids. All Salmonella enteritidis isolated carried a single plasmid of 36 Mdal and were resistant to guinea pig serum; one strain that was tested was virulent.

In a recent study in the United Kingdom (61) virulence of Salmonella typhimuruim and Salmonella dublin were attributed to the presence of large plasmids.

In a joint study (119) by workers in Finland and the United States, plasmid associated virulence of Salmonella enteritidis was reported.

In 1986 a study indicated (37) that the use of cloned, random chromosomal sequences as probes to identify Salmonella species demonstrated distinctive profiles.

In a study in the United States (114) Salmonella enteritidis was isolated from yolk, dead-in-shell eggs from breeder hens and ovaries from layers. Restriction endonuclease finger prints of the plasmids and genomic DNA of these isolates indicated that there are distinct differences between the various isolates.

A study in Bulgaria (31) indicated that erythrocyte antigen was far superior to routinely used color test antigen in terms of time consumed, intensity, and number of reacting birds to Salmonella enteritidis.

In a 1983 study in Bulgaria (59), sheep erythrocytes were sensitized with a polysaccharide LPS preparation of Salmonella gallinarum or Salmonella enteritidis, and were stabilized by adding glutaraldehyde. This antigen, used in the passive haemagglutinatin test, was more sensitive than a standard pullorum disease antigen used in agglutinatin tests on the same serum samples.

Swedish workers (19) studied indirect IFA for detection of Salmonella enteritidis in outbreaks using direct fecal smears, enriched broth cultures and agar-grown colonies. Their success rate was 75%, 89% and 100%. Their detection of serological responses by ELISA was 92% positive with LPS antigen.

In the United States (117) workers reported on a practical Salmonella environmental sampling procedure for poultry farms utilizing an enzyme immunoassay, antigen capture technology.

VACCINES, KILLED & LIVE

Live vaccines in mice (70) proved better than killed vaccines in India in 1979. Live vaccines with complete adjuvant induced good protection. Cross-protection could be induced with the live vaccine with complete adjuvant against Salmonella enteritidis infection.

Several workers (72) have studied live vaccines with and without complete adjuvants with Salmonella enteritidis in Hungary.

In a study in New York (73) mice vaccinated with live Salmonella enteritidis rapidly developed an effective antibacterial resistance to intragastric challenge with virulent Salmonella enteritidis. Heat-killed saline suspensions (200 mu g, dry wt) of Salmonella enteritidis or Salmonella pullorum were unable to induce an effective antimicrobial resistance to subsequent challenge. However, when the organisms were suspended in Freund complete adjuvant, both vaccines induced resistance to i/v and intragastric challenge.

In India, (79) investigators, in 1982, found that immunization with live Salmonella enteritidis generated delayed hypersensitivity and activated macrophages, while immunization with formalized vaccine generated neither.

Another study in India (80) in 1980 determined that the degree of clearance varied with vaccine, but mice vaccinated with Salmonella typhimurium M206 and Salmonella enteritidis Se 795 live vaccines plus adjuvant cleared challenge organism by 14 days after challenge.

Workers in Virginia Commonwealth University at Richmond (75), believed that humoral elements play an important role in acquired immunity in murine salmonellosis by opsonization of the pathogen.

New York investigators at Trudeau Institute (83) found that the highest dose of 500 mu g of dead cells in adjuvant induced an immune response equivalent to that seen in actively infected mice.

They also determined (84) that heat-killed vaccine given orally in mice delayed systemic emergence. Live vaccine developed an effective immunity against subsequent infections.

A further study in India (86) indicated that cross protection against Salmonella enteritidis is shown in mice with live vaccines consisting of Salmonella enteritidis, Salmonella typhimurium and Salmonella gallinarum.

In the Journal of Medical Microbiology in 1986 (90) it was reported that live vaccine infection produced cross-reacting immune cell response in mice.

A vaccine preparation (93) was patented and described that is useful for peroral administration to mice; chickens were not tested.

Japanese investigators in 1985 (95) report the immunizing capacity of Salmonella enteritidis AL1192, a strain that has been cured of a 36-megadalton plasmid, to protect ddY mice against subsequent challenge with virulent salmonellas. This strain, which was given subcutaneously at a dose of 10 to the 6 organisms, provided significant protection against oral, subcutaneous, or intraperitoneal challenge by virulent wild-type strains of not only Salmonella enteritidis, but also Salmonella dublin, Salmonella naestved, and Salmonella typhimurium. Workers in Idaho reported that (97) outer membrane proteins (OMP), extracted from Salmonella enteritidis, Salmonella anatum, Salmonella typhimurium, and Salmonella infantis, were cross-linked to form a large immunogen (4-OMP-lipopolysaccharide [LPS). ...Cross-linked OMP, without the inclusion of muramyl dipeptide, may have potential as vaccine components and may induce immunologic memory.

Japanese researchers (99) indicated that Klebsiella 03 lipopolysaccharide (LPS) exhibits strong adjuvant activity in augmenting antibody response against protein antigens in mice. They claimed that LPS from certain Klebsiella (example Klebsiella serotype 03) posses Mannans which is a strong adjuvant.

Idaho workers found that (101) bacterin immunity to Salmonella enteritidis may last for 130 days in mice.

Efficiacy of live Salmonella enteritidis (103) vaccine, gave good protection in mice by workers in Japan.

Work done by George, et. al., in 1987 (98) presents evidence on the use of live vaccines for Salmonella enteritidis in mice models. The author compared the use of live vaccine with a sonicate from Salmonella enteritidis. The live vaccine appeared better than the sonicate. The live vaccine induced both CMI and DTH responses.

A study by Idaho workers (105) indicates that Interleukin I may have an apparent role in the enhancment of immune response to OMP-LPS Salmonella antigens. Salmonella enteritidis was among four serotypes used in this trial.

A European study in 1980 (106) indicated that the Salmonella enteritidis proteins induced antibodies against a specific Salmonella enteritidis protein, in addition to numerous heterologous determinants. It suggests

some reason for optimism that vaccines for Salmonella enteritidis may result in specific immunity to prevent infection, and that fractionated vaccines may be efficious, reducing the risk of reversion of live attenuated strains.

Israeli workers discovered in 1977 **(85)** that vaccination of 10-day-old chickens with a live, relatively avirulent Salmonella gallinarum strain induced antibodies against the main antigens of the pathogen, namely its proteins. Precipitins in agar gel were obtained against the free proteins and the protein conjugated in the somatic antigen of Salmonella gallinarum and against antigens from the related Salmonella enteritidis. Antipolysaccharide antibodies were induced in small quantities which were however sufficient to allow their identification with the agar gel diffusion technique.

Workers in New York State **(78)** presumed that the local gut defenses were responsible for large differences in the lethal effects of an oral compared with parenteral challenge.

Hemopoietic responses to viable salmonellae of mice **(66)** immunized with viable bacteria differed from the responses to killed salmonellae of normal, non-immunized mice in a study in Japan. These results suggest that both LPS and cell-mediated immune responses affect hemopoietic stem cells in mice infected with salmonellae.

Canadian workers in 1972 **(82)** found that chicks two, three and four weeks of age respond well serologically to endotoxin given intravenously, with the older chicks giving the best response.

Another patent, **(94)** a vaccine comprised of mutant Salmonella enteritidis, a B-lymphocyte proliferating immunostimulant, a protein, and lipid-binding carrier is useful for immunizing mammals and birds against diseases caused by enterotoxin-producing Gram-negative bacteria. The immune modulator described is a detoxified extract of lipopolysaccharide and is useful in combination with many antigens to enhance the primary immune response. Work done in Germany **(71)** in 1981 provides suggestions of efficacy of oral vaccination that could be useful in study design in chicks.

Workers in India **(104)** reported on the use of Salmonella enteritidis and Salmonella gallinarum bacterins as immunizing agents in chicks.

University of Minnesota workers **(115)** reported in 1988 that they were successful in using Formalin-inactivated and heat and acetone killed vaccines composed of outer membrane proteins to clear the organism after challenge in turkeys. Clearance of systemic infection was interpreted as a measure of protection.

COMPETITIVE EXCLUSION

University of Massachusetts workers in 1979 **(67)** found that the individual-bird challenge test system appeared to yield a more precise measurement of protection than the seeder-bird system and indicated that trypticase soy broth is as effective as VL broth for anaerobic culture of the protective microflora.

These same workers in 1981 **(68)** determined that lactobacilli as a prevention reduced the number of salmonellae adhering to the crop mucosa by 1 to 2 logs. Treatment with lactobacilli did not lower the number of chickens shedding salmonellae or reduce the number of salmonellae adhering to the mucosa of the cecum.

In 1982 they also reported that **(69)** groups of chicks treated with a native protective microflora were infrequently colonized by Salmonella serotypes. Differences in colonization are explainable by lack of competing bacteria in the monocolonized group and by various degrees of protection provided by microflora colonizing the other groups. Once vertical transmission has been interrupted, control strategies may need to be in place to prevent horizontal spread; competitive exclusion may be a significant tool.

In 1988 workers in Sweden and Finland **(118)** evaluated the Salmonella controlling effects of the nationwide use of a competitive exclusion (CE) culture in broilers. Only one of 144 flocks previously contaminated was found to be subsequently contaminated with Salmonella.

ANTIBIOTIC RESISTANCE

In a study in West Germany **(76)** of 2713 Salmonella strains isolated by veterinary laboratories in 1974, 21.7% were resistant to one or several of the antibacterial agents.

Bulgarian investigators **(81)** in 1980 studied a total of 326 Salmonella strains isolated from poultry between 1969 and 1979; including 34 Salmonella enteritidis isolates. The results indicate that chloramphenicol and furazolidone can be used for the control of salmonellosis in poultry, either singly or in combination. Good results could also be expected with gentamicin, kanamycin and carbenicillin.

Another German study **(89)** indicated that the resistance patterns showed considerable variance over the time of observation. All types of resistance appeared much more frequently than expected from calculation of possible gene-bound properties; the only exception was the double resistance to cephalosporine and tetracycline which corresponds with genetic laws. The authors compare 1982-83 data against 1980-81 data on the frequency of single resistance marker decreases while multiple resistant markers increased during same period.

In 1986 another Bulgarian study (91) reported that the percentage of Salmonella species isolated from chickens that demonstrate resistance to antibiotics commonly were used for prophylactic and control purposes. Differences were found in the resistance of the various species of Salmonella also during the year with no definite trend toward a rise of the resistant strains.

GENETIC RESISTANCE

In a study in Chicago (74) in 1973, mice have been sucessfully selected for genetic resistance to Salmonella enteritidis.

Workers in the United Kingdom in 1979 (77) found that it is possible to select mice for genetic resistance to a very virulent strain of Salmonella enteritidis.

ENVIRONMENTAL SURVIVAL

Workers at Cornell University (22) found that washing with a chlorine sanitizer (under commercial conditions) was highly effective in reducing surface bacterial counts on egg shells. In this trial Salmonella enteritidis was detected on dirty eggs shells in 4 of 6 farms. Salmonella enteritidis and Salmonella hadar were recovered from washed, nest clean and dirty eggs in 2 of 6 farms. Proper egg washing and confinement of duck breeders should minimize the problem of salmonellosis in ducklings. Salmonella enteritidis was found in May, 1983 to be a frequent contaminant on the surface of commercial duck eggs from 2 of 6 farms. Proper washing of eggs with a chlorine sanitizer was highly effective in reducing the bacterial counts on shell eggs.

A Taiwan study in 1987 (15) provides information on environmental survival times in relationship to future clean-up of contaminated premises, comments on the survivability of Salmonella enteritidis in the environment as posing a threat to Public Health.

In Poland (35) it was determined that prefermented and dehydrated sewage sediments frequently contain Salmonella (43 of 167 samples, or 25.7%) and Salmonella enteritidis was among the more frequent isolates.

Workers at Purdue University (100) found the survival times of different Salmonella serotypes in artificially inoculated pig feed or rendered meat-bone meal at 21-24degC varied: Salmonella typhisuis 34 days in feed, Salmonella choleraesuis 92 days in meal and 436 days in feed, Salmonella anatum 299 days in meal and 429 days in feed, Salmonella typhimurium 539 days in meal, Salmonella infantis 588 days in meal and 723 days in feed, and Salmonella enteritidis 730 days in feed and 750 days (maximum observation period) in meat.

CONTROL PROGRAM & MANAGEMENT

Maryland initiated (87) a statewide chicken testing program for Salmonella in egg producers.

In Georgia, to study improved methods (92) for converting waste foods into safe sources of livestock feed while also conserving energy, the antimicrobial effects of Lactobacillus fermentation of edible waste contaminated with infected carcasses was examined. The pH also decreased most rapidly during this time, and was followed by a leveling off. Salmonella enteritidis survived 5 days at 20 degrees Centigrade, 1 day at 30 degrees Centigrade and was not isolated from the 40 degrees Centigrade samples after 24 hours. Results indicated that Lactobacillus fermentation is an alternative method of treating edible wastes.

New York workers (88) conclude that proper egg washing and confinement of duck breeders should minimize the problem of salmonellosis in ducklings.

Model programs for routine monitoring and surveillance for Salmonella enteritidis are being formulated by USDA and FDA through the work of the Interagency Task Force.

FOOD SCIENCE AND PUBLIC HEALTH

A CDC report (107) stated that transatlantic outbreaks are most often the result of an improper temperature for preparation or for holding food in the flight kitchens. Serving the flight crew meals from one kitchen carries the risk that the entire crew will become ill. The Salmonella source was from outside the United States in that report.

Salmonella enteritidis (108) appears to be sensitive to sorbates according to a study done in the Institute of Food Technology in Chicago in 1984.

In Czechoslovakia in 1983 (109) inactivation of salmonellae during drying of bulk egg was investigated, dependence on temperature of drying air and throughput of the drier being considered. Trials were conducted with raw or pasteurized bulk egg; a mixture of Salmonella typhimurium, Salmonella panama and Salmonella enteritidis was used. On the basis of the results, it is concluded that the log reduction in Salmonella count ranges from 1.60 to 2.80 depending on drying conditions, and that inactivatin of salmonellae increased with increasing drying air temperature over the range studied (70-90 degree C).

In a USDA (110) study at Beltsville, Maryland it was found that heat treatment of 3.5 minute at 60 degree C for liquid whole egg homogenates inoculated with a heavy concentration (10 to the 9 per ml. of Salmonella enteritidis serotypes resulted in either survival of a small

number of bacteria as shown by the occurrence of extensive tailings of survivor curves, or by recovery of dead bacteria after incubating in trypticase soy broth for 24 h. Exposure at 60 degree C for as long as 6, 10, 60, and 180 min for serotype derby, typhimurium (var. Copenhagen), Newport, and Senftenberg, respectively, yielded a small number of survivors. No survivors were detected for serotype anatum following 1.50 min at 60 degree C. The importance of recovery tests is emphasized.

In Germany in 1975 **(111)** the incidence and origin of contamination of foods with Salmonella enteritidis, and the effectiveness of legal control were discussed. It is emphasised that contamination originationg at an earlier stage of food processing may appear later through carriers. In order to prevent contamination close cooperation with veterinary control measures against zoonoses is required. Ingestion of >1.25 x 105 to 4.4 x 107 live bacteria is necessary before appearance of symptoms, emphasizing the importance of storage and handling under conditions minimizing the growth of isolated organisms.

A Belgium study in 1984 **(112)** presented several enrichment medias for culture and isolation methodology from salmonella from foods, especially.

In Spain, in 1982, a study done on cheese **(113)** found that of 8 enrichment procedures examined, the most efficient was selective enrichment in selenite cystine broth followed by streaking on bismuth sulphite agar, which gave recovery of salmonellae from 93% of positive samples.

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